

[7] Another challenge to the broad utilization of immuno-oncology with respect to T-cell engaging bispecific molecules is the availability of suitable targets (Bacac et al., Clin Cancer Res; 22(13) July 1, 2016). For example, solid tumor targets may be overexpressed on tumor cells but expressed at lower, yet significant levels on non-malignant primary cells in critical tissues. In nature, according to Bacac et al, T cells can distinguish between high- and low-antigen expressing cells by means of relatively low-affinity T cell receptors (TCRs) that can still achieve high-avidity binding to target cells expressing sufficiently high levels of target antigen. T-cell engaging bispecific molecules that could facilitate the same, and thus maximize the window between killing of high- and low-target expressing cells, are thus highly desirable. One approach discussed in the art is the use of dual targeting of two antigens on the same cell leads to improved target selectivity over normal tissues that express only one or low levels of both target antigens. This effect is thought to be dependent on the avidity component mediated by the concurrent binding of the bsAb to both antigens on the same cell. With respect to dual targeting as such, some multispecific monoclonal antibodies (mAb) or other immune constructs are known in the art. WO 2014/116846 teaches a multispecific binding protein comprising a first binding site that specifically binds to a target cell antigen, a second binding site that specifically binds to a cell surface receptor on an immune cell, and a third binding site that specifically binds to cell surface modulator on the immune cell. US 2017/0022274 discloses a trivalent T-cell redirecting complex comprising a bispecific antibody, wherein the bispecific antibody has two binding sites against a tumor-associated antigen (TAA) and one binding site against a T-cell. While different multispecific antibodies or antibody fragments are known in the art, some of which address T-cells, no CD20 and CD22 targeting bispecific molecules employing the mechanism of a -preferably single chain-bispecific T-cell engaging molecule has been proposed before which both addresses the need of overcoming antigen loss/tumor escape and to reduce dose-limiting toxicity in antibody-based immunotherapeutics while effectively redirecting T-cells by one stable and ready-to-use therapeutic system.

Summary

[8] In view of the needs described above, it is an object of the present disclosure to provide CD20 and CD22 targeting antigen-binding molecules, typically polypeptides, such as T cell engaging bispecific molecules, which are specifically suitable to bind two antigens on a target cell associated with specific conditions and one antigen on an effector cell at the same time, preferably for use in the treatment of said specific conditions. The molecules should further show high producibility, stability and activity. Accordingly, the present disclosure provides a CD20 and CD22 targeting bispecific antigen-binding molecule characterized by comprising a first domain binding to CD20 as the first target cell surface antigen (TAA), a second domain binding to the CD22 (the second TAA), a third domain binding to an extracellular epitope of the human and non-human, e.g. *Macaca* CD3ε chain, and preferably a fourth domain, which is a specific Fc modality which modulates half-life of the molecule. Preferably, the domains are binding domains comprised of VH and VL domains in amino to carboxyl orientation, respectively, wherein a flexible but short peptide linker links the VL of the first

binding domain to the VH of the second binding domain. Surprisingly, activity of the molecules of the present disclosure against target cells associated with particular diseases can be preserved thereby without steric hindrance between the first and the second binding domain, and without the requirement of providing long linkers which would disadvantageously be more prone to degradation, cleavage or the like than the instantly provided shorter linkers. At the same time, the molecules are well producible and show good product homogeneity. Moreover, the disclosure provides a polynucleotide encoding the antigen-binding molecule, a vector comprising this polynucleotide, and host cells expressing the construct and a pharmaceutical composition comprising the same.

[9] In a first aspect, it is envisaged in the context of the present disclosure to provide a

10 CD20 and CD22 targeting antigen-binding molecule comprising at least three binding domains, wherein

(i.) the first binding domain comprises a paratope which immuno-specifically binds to CD20, wherein the first binding domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:

15 a) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 - 63,

b) CDR H1-3 of SEQ ID NO: 71 - 73 and CDR L1-3 of SEQ ID NO: 74 - 76,

c) CDR H1-3 of SEQ ID NO: 84 - 86 and CDR L1-3 of SEQ ID NO: 87 - 89, and

d) CDR H1-3 of SEQ ID NO: 97 - 99 and CDR L1-3 of SEQ ID NO: 100 - 102;

20 (ii.) the second binding domain comprises a paratope which immuno-specifically binds to CD22, wherein the first binding domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from

a) CDR H1-3 of SEQ ID NO: 138 - 140 and CDR L1-3 of SEQ ID NO: 141 - 143,

b) CDR H1-3 of SEQ ID NO: 151 - 153 and CDR L1-3 of SEQ ID NO: 154 - 156,

c) CDR H1-3 of SEQ ID NO: 164 - 166 and CDR L1-3 of SEQ ID NO: 167 - 169,

25 d) CDR H1-3 of SEQ ID NO: 177 - 179 and CDR L1-3 of SEQ ID NO: 180 - 182,

e) CDR H1-3 of SEQ ID NO: 190 - 192 and CDR L1-3 of SEQ ID NO: 193 - 195,

f) CDR H1-3 of SEQ ID NO: 203 - 205 and CDR L1-3 of SEQ ID NO: 206 - 208,

g) CDR H1-3 of SEQ ID NO: 125 - 127 and CDR L1-3 of SEQ ID NO: 128 - 130,

h) CDR H1-3 of SEQ ID NO: 216 - 218 and CDR L1-3 of SEQ ID NO: 219 - 221, and

30 i) CDR H1-3 of SEQ ID NO: 379 - 381 and CDR L1-3 of SEQ ID NO: 382 - 384;

and

(iii.) the third binding domain comprises a paratope which immune-specifically binds to an extracellular epitope of the human and/or the Macaca CD3ε chain,

wherein the first, second and third binding domain are arranged in an amino to carboxyl order, and wherein the first binding domain and the second binding domain are linked by a peptide

5 linker having a length of 5 to 24, preferably 18 amino acids.

[10] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the antigen-binding molecule comprises a fourth domain which comprises two polypeptide monomers, each comprising a hinge, a CH2 and a CH3 domain, wherein said two polypeptide monomers are fused to each other via a peptide linker.

10 **[11]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein said forth domain comprises in an amino to carboxyl order:

hinge-CH2-CH3-linker-hinge-CH2-CH3.

15 **[12]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein each of said polypeptide monomers in the fourth domain has an amino acid sequence that is at least 90% identical to a sequence selected from the group from the group consisting of: SEQ ID NO: 17-24, wherein preferably each of said polypeptide monomers has an amino acid sequence selected from SEQ ID NO: 17-24.

20 **[13]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the CH2 domain comprises an intra domain cysteine disulfide bridge.

[14] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the first, second, third and the optional fourth binding domain are arranged in an amino to carboxyl order.

25 **[15]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the antigen-binding molecule is a single chain antigen-binding molecule, preferably a multispecific scFv antigen-binding molecule.

30 **[16]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the first, second, and third binding domain each comprise in a amino to carboxyl order a VH domain and a VL domain.

[17] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the peptide linker between the VL of the first binding

domain and the VH of the second binding domain is selected from having a length of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 amino acids, preferably 5, 6, 7, 8, 9, 10, 11 or 12 amino acids, more preferably 6 amino acids.

[18] Within said aspect, it is also envisaged in the context of the present disclosure to provide an
 5 multispecific antigen-binding molecule, wherein the peptide linker between the VL of the first binding domain and the VH of the second binding domain is a flexible linker which comprises serine and glycine as amino acid building blocks, preferably only serine (Ser, S) and glycine (Gly, G).

[19] Within said aspect, it is also envisaged in the context of the present disclosure to provide an
 10 multispecific antigen-binding molecule, wherein the peptide linker between the first binding domain and the second binding domain is preferably rich in small and/or hydrophilic amino acids and preferably selected from the group consisting of S(G₄S)_n, (G₄S)_n, (G₄)_n, and (G₅)_n, wherein n equals 1, 2, 3 or 4, more preferably n equals 1 or 2, more preferably SG₄S.

[20] Within said aspect, it is also envisaged in the context of the present disclosure to provide a
 CD20 and CD22 targeting antigen-binding molecule, wherein

15 the first binding domain and the second binding domain each comprise a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:

a) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding
 20 domain and CDR H1-3 of SEQ ID NO: 138 - 140 and CDR L1-3 of SEQ ID NO: 141 – 143 of the second binding domain;

b) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding
 domain and CDR H1-3 of SEQ ID NO: 151 - 153 and CDR L1-3 of SEQ ID NO: 154 – 156 of the second binding domain;

c) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding
 25 domain and CDR H1-3 of SEQ ID NO: 164 - 166 and CDR L1-3 of SEQ ID NO: 167 - 169 of the second binding domain;

d) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding
 domain and CDR H1-3 of SEQ ID NO: 177 - 179 and CDR L1-3 of SEQ ID NO: 180 – 182 of the second binding domain;

30 e) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding domain and CDR H1-3 of SEQ ID NO: 190 - 192 and CDR L1-3 of SEQ ID NO: 193 – 195 of the second binding domain;

- f) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding domain and CDR H1-3 of SEQ ID NO: 203 - 205 and CDR L1-3 of SEQ ID NO: 206 - 208 of the second binding domain;
- 5 g) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding domain and CDR H1-3 of SEQ ID NO: 125 - 127 and CDR L1-3 of SEQ ID NO: 128 – 130 of the second binding domain;
- h) CDR H1-3 of SEQ ID NO: 58 - 60 and CDR L1-3 of SEQ ID NO: 61 – 63 of the first binding domain and CDR H1-3 of SEQ ID NO: 216 - 218 and CDR L1-3 of SEQ ID NO: 219 – 221 of the second binding domain;
- 10 i) CDR H1-3 of SEQ ID NO: 71 - 73 and CDR L1-3 of SEQ ID NO: 74 – 76 of the first binding domain and CDR H1-3 of SEQ ID NO: 379 - 381 and CDR L1-3 of SEQ ID NO: 382 – 384 of the second binding domain;
- j) CDR H1-3 of SEQ ID NO: 71 - 73 and CDR L1-3 of SEQ ID NO: 74 – 76 of the first binding domain and CDR H1-3 of SEQ ID NO: 203 - 205 and CDR L1-3 of SEQ ID NO: 206 - 208 of the second binding domain;
- 15 k) CDR H1-3 of SEQ ID NO: 84 - 86 and CDR L1-3 of SEQ ID NO: 87 – 89 of the first binding domain and CDR H1-3 of SEQ ID NO: 164 - 166 and CDR L1-3 of SEQ ID NO: 167 – 169 of the second binding domain;
- l) CDR H1-3 of SEQ ID NO: 97 - 99 and CDR L1-3 of SEQ ID NO: 100 – 102 of the first binding domain and CDR H1-3 of SEQ ID NO: 177 - 179 and CDR L1-3 of SEQ ID NO: 180 – 182 of the second binding domain;
- 20 m) CDR H1-3 of SEQ ID NO: 97 - 99 and CDR L1-3 of SEQ ID NO: 100 – 102 of the first binding domain and CDR H1-3 of SEQ ID NO: 190 - 192 and CDR L1-3 of SEQ ID NO: 193 – 195 of the second binding domain.
- 25 **[21]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the first binding domains is capable of binding to the first target cell surface antigen CD20 and the second binding domain is capable of binding to the second target cell surface antigen CD22 simultaneously, preferably wherein the first target cell surface antigen and the second target cell surface antigen are on the same target cell.
- 30 **[22]** Within said aspect, it is also envisaged in the context of the present disclosure to provide a CD20 and CD22 targeting antigen-binding molecule of claim 1, wherein the third binding domain comprise a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:
- a) CDR H1-3 of SEQ ID NO: 392 - 394 and CDR L1-3 of SEQ ID NO: 395 – 397; and

b) CDR H1-3 of SEQ ID NO: 401 - 403 and CDR L1-3 of SEQ ID NO: 404- 406.

[23] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the first, second and third domain, which are fused by
5 respective peptide linkers, are fused to the fourth domain via a peptide linker.

[24] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the antigen-binding molecule comprises in an amino to carboxyl order:

- (a) the first domain;
- 10 (b) a peptide linker preferably having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-4 and 9-12, preferably 11;
- (c) the second domain;
- (d) a peptide linker preferably having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3; and
- 15 (e) the third domain.

[25] Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the antigen-binding molecule further comprises in an amino to carboxyl order:

- (f) a peptide linker having an amino acid sequence selected from the group consisting of
20 SEQ ID NOs: 1, 2, 3, 9, 10, 11 and 12;
- (e) the first polypeptide monomer of the fourth domain;
- (f) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7 and 8; and
- (g) the second polypeptide monomer of the fourth domain.

[26] Within said aspect, it is also envisaged in the context of the present disclosure to provide an antigen-binding molecule, wherein the first binding domain comprises a VH region and a VL region selected from SEQ ID Nos: 64 as VH and 65 as L , 77 as VH and 78 as VL, 90 as VH and 91 as VL, 103 as VH and 104 as VL, respectively, and wherein the second binding domain comprises a VH

region and a VL region selected from SEQ ID Nos: 144 as VH and 145 as VL, 157 and 158, 172 and 173, 183 and 184, 196 and 197, 209 and 210, 131 and 132, and 385 and 386, respectively.

5 **[27]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an antigen-binding molecule, wherein the first binding domain comprises a scFv sequence selected from the group consisting of SEQ ID Nos: 66, 79, 92, and 105, and wherein the second binding domain comprises a scFv sequence selected from the group consisting of SEQ ID Nos 146, 159, 172, 185, 198, 211, 133, 224 and 387, respectively.

10 **[28]** Within said aspect, it is also envisaged in the context of the present disclosure to provide an multispecific antigen-binding molecule, wherein the antigen-binding molecule comprises a first (CD20) and second (CD22) target binding domain together with a third effector (CD3) binding domain and a fourth domain conferring extended half-life, the three binding domains and the fourth domain linked together having a sequence selected from the group consisting of SEQ ID Nos: 238, 248, 258, 268, 278, 288, 308, 318, 328, 338, 348, 368 and 378.

15 **[29]** In a second aspect, it is further envisaged in the context of the present disclosure to provide a polynucleotide encoding an antigen-binding molecule of the present disclosure.

[30] In a third aspect, it is also envisaged in the context of the present disclosure to provide a vector comprising a polynucleotide of the present disclosure.

20 **[31]** In a fourth aspect, it is further envisaged in the context of the present disclosure to provide a host cell transformed or transfected with the polynucleotide or with the vector of the present disclosure.

[32] In a fifth aspect, it is also envisaged in the context of the present disclosure to provide a process for the production of an antigen-binding molecule of the present disclosure, said process comprising culturing a host cell of the present disclosure under conditions allowing the expression of the antigen-binding molecule and recovering the produced antigen-binding molecule from the culture.

25 **[33]** In a sixth aspect, it is further envisaged in the context of the present disclosure to provide a pharmaceutical composition comprising an antigen-binding molecule of the present disclosure or produced according to the process of the present disclosure.

[34] Within said aspect, is also envisaged in the context of the present disclosure that the pharmaceutical composition is stable for at least four weeks at about -20°C.

30 **[35]** It is further envisaged in the context of the present disclosure to provide the antigen-binding molecule of the present disclosure, or produced according to the process of the present disclosure, for use in the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, cancer or an immunological disorder. In one aspect, the present disclosure refers to the CD20 and CD22 targeting antigen-binding molecule as disclosed herein, or produced according to
35 the process as disclosed herein, for use in medicine. In still another aspect, the present disclosure refers to the use of the CD20 and CD22 targeting antigen-binding molecule as disclosed herein, or produced according to the process as disclosed herein in the manufacture of a medicament for the treatment or amelioration of a proliferative disease, a tumorous disease, cancer, or an immunological disorder, wherein the disease preferably is Non-Hodgkin lymphoma (NHL), Non-small-cell lung carcinoma (NSCLC) and Colorectal cancer (CRC).
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[36] Within said example, it is also envisaged in the context of the present disclosure that the CD20xCD22 targeting antigen-binding molecule is for use in the treatment of Non-Hodgkin lymphoma.

DESCRIPTION OF THE FIGURES

5 **Figure 1:** 48-hour FACS-based cytotoxicity assay of CD20- and CD22 dual targeting antigen-binding molecules with human CD20 and CD22 double positive human cell line Oci-Ly 1 (A), human CD20 single positive human cell line Oci-Ly 1 (CD22 knock out clone #A1) (B), and CD22 single positive human cell line Oci-Ly 1 (CD20 knock out clone #A5) (C) as target cells and panT as effector cells (E:T ratio 10:1). EC50 values are determined by the four parametric logistic regression models for
10 evaluation of sigmoid dose response curves with fixed hill slope. **Detailed Description**

[37] In the context of the present invention, a CD20 and CD22 targeting antigen-binding molecule is provided comprising at least three binding domains, wherein the first and second binding domain in amino to carboxyl orientation are capable to preferably target CD20 and CD22 simultaneously, wherein the third binding domain binds to an extracellular epitope of the human and/or the Macaca
15 CD3ε chain on an effector cell which is a T cell.

[38] It is a surprising finding in the context of the present invention that the T-cell engaging CD20 and CD22 targeting antigen-binding molecules according to the present invention with selected combinations of CD20 and CD22 target binders show superior yield, stability and a balanced activity between the two target binders. This improves both the practical aspects of producibility and storage
20 capabilities as well as preferably reliable drug action. In this regard, it is found that molecules according to the present invention show HIC elution slopes as demonstrated herein which are typically higher than 15, preferably higher than 20 or even 25. Molecules according the generic setup according to the present invention which, however, do not comprise the specific binder selection as described herein, do typically show lower values indicating less product homogeneity. Even more pronounced is
25 the yield as an indication for the overall productivity which is typically above 10 mg/L, preferably above 15 or even 20 mg/L of monomer, i.e. desired product. In contrast, other molecules of the generic format underlying the molecules described herein typically do not reach a yield above 10 mg/L. As a further indicator of product quality, the monomer peak symmetry in size exclusion chromatography (SEC) is typically improved for molecules comprising the specific binder selection according to the
30 present invention. Such peak symmetry is preferably below a value of 1.4, more preferably below a value of 1.35 or lower. As the skilled person is aware of, a value close to 1 is typically preferred. However, other molecules according to the generic format underlying the molecules of the present invention typically to not reach values below 1.4. Further, molecules of the present invention typically show good activity with respect to cells which express both targets CD20 and CD22. Therefore,
35 observed EC50 values are typically surprisingly low for molecules comprising the specific selection of anti-CD20 and anti-CD22 binders as claimed herein. Accordingly, the molecules of the present